COLLECTION OF ANIMAL VIRUSES and ANTISERA, CHLAMYDIAE and RICKETTSIAE

ATCC® Catalog No. VR-1516
Adenovirus Type 5 Reference Material [1]
Produced by ARMWG

Description and Background

The Adenovirus Reference Material (ARM) consists of purified Adenovirus, Type 5 (wild type adenovirus, see ATCC VR-5) formulated as a sterile liquid in 20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0 at room temperature, and stored frozen at –70°C. The configuration is 0.5-mL in a Type II glass vial with a Teflon-coated gray butyl stopper, plus an aluminum seal and crimp closure. The U.S. Food and Drug Administration (FDA) has made recommendations for the use of the ARM when characterizing adenoviral gene therapy products [2].

The ARM was developed under the guidance of the Adenovirus Reference Material Working Group (ARMWG) and the U.S. FDA through the donation of services and supplies by a large number of laboratories and organizations from the United States, Canada, France, the Netherlands, Germany, and the United Kingdom [1, 3]. All information regarding the development and characterization of the ARM can be found on The International Society for BioProcess Technology website at http://www.isbiotech.org/. The purpose of the ARM is to define the particle unit and infectious unit for adenovirus-based gene vectors and establish a reference point for comparisons. The NIH Recombinant DNA Advisory Committee recommended the development of such a reference-testing agent in their report issued January 2002 [4].

Assigned Concentration and Titer and Expiry

The ARMWG assigned the particle concentration and infectious titer based on statistical analyses [5, 6] of the data derived during the characterization phase of the project. Procedures for obtaining and analyzing these data were provided by the ARMWG (http://www.isbiotech.org/). The particle concentration is 5.8×10^{11} particles/mL, with 95% certainty that the true particle concentration lies within the range of 5.6×10^{11} to 6.0×10^{11} particles/mL. The infectious titer on HEK 293 cells is 7×10^{10} NAS Infectious Units (NIU)/mL, with 95% certainty that the infectious titer on HEK 293 cells lies within the range of 7×10^{10} to 8×10^{10} NIU/mL.

The particle concentration was assigned based on data submitted by 13 different laboratories, each of which performed an A 260nm/SDS procedure that was provided by the ARMWG. The ARMWG procedure required two vials of ARM, and was conducted on 4 dilutions in triplicate. Fourteen data points resulted. If the ARMWG procedure for particle determination is performed with the ARM, 99.7% of the time the result should fall within the range of 4.8×10^{11} to 6.9×10^{11} particles/mL (within 3 standard deviations of the mean).

The infectious titer was assigned based on data submitted by 17 different laboratories, each of which assayed two independent dilutions series on HEK 293 cells in a procedure provided by the ARMWG. The procedure was designed to provide many data points, in contrast to assays that are designed for the ruggedness and throughput typical of a Quality Control laboratory. A square-root-of-two-fold dilution series was utilized, and the end point was cytopathic effect (CPE) read after 10 days. The ARMWG infectious titer procedure incorporated a correction for the slow diffusion of the adenovirus particle in solutions into the titer calculation from the raw data, *i.e.*, the NAS calculation method [7]. Thirty valid assay data sets were incorporated into the analysis. If the ARMWG procedure for infectious titer is performed with the ARM, 99.7% of the time the result should fall within the range of 3×10^{10} to 18×10^{10} NAS IU/mL (within 3 standard deviations of the mean). The precision of the ARMWG procedure is such that data should be reported as a single significant figure.

The ARM was not assigned an expiration date at the time of manufacture but has been monitored for stability long-term. The material is stable when stored long-term at -80ºC (see Table 2).

Other Available Characterization Information

Characterization data are for Lot No. 001503. The complete DNA sequence of the Adenovirus Reference Material genome was determined and is deposited with GenBank [8]. The ARM GenBank accession number is AY339865. The sequence is similar, but not identical, to the adenovirus 5 sequence that can be found within GenBank at accession number NC 001406.

The ARM was analyzed for a variety of impurities including the amount and fragment size distribution of residual HEK 293 host cell DNA via quantitative real-time PCR [9]. Less than 3 pg of residual host cell DNA was found per µg of total DNA for 293 cell DNA fragments sizes of 120, 411, and 757 base pairs. Using a commercial ELISA kit [10], residual HEK 293 host cell proteins were found to be 18 ng/mL. Residual BSA was present at <0.5 ng/mL. Free hexon was assessed by an immunoaffinity/gel filtration assay, and was determined to

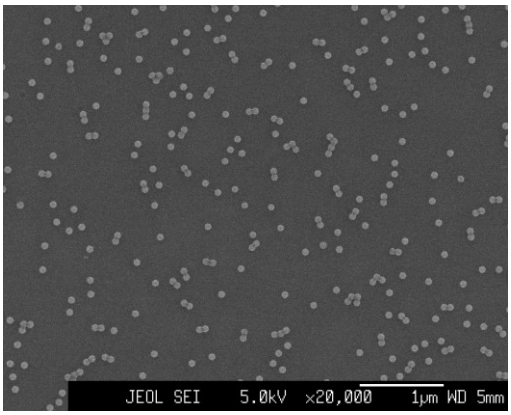
be 1.16 µg/mL, or approximately 2.0 µg free hexon per 10^{12} adenoviral particles. The Adenovirus Reference Material was analyzed by a published reverse phase HPLC assay [11], and no 31 K MW precursor protein was detected. Endotoxin was <0.15 EU/mL. The A 260nm to A 280nm ratio in the presence of 0.1% (w/v) SDS was 1.37.

The ARM was tested and found to be free of adventitious agents.

Particle size distribution was assessed by photon correlation spectroscopy (PCS). Data from several laboratories demonstrated that the preparation is very homogenous in nature, consisting primarily of single particles.

VR-1516 was analyzed using field emission scanning electron microscopy (FESEM) image analysis to characterize mean particle diameter, perimeter, and multiplet order. The total estimated mean diameter of the single particles was 86.2 ± 5.37 nm. Particle perimeter distribution analysis was applied to look at micro-aggregation with VR-1516. The analysis revealed that the material consisted predominantly (>70%) of icosahedral shaped, single adenovirus particles. The remaining virus population was composed of doublets, triplets, or multiplets with more than three adenovirus particles.

Data for ARM lot numbers 001504, 001505, and 001506 are shown in Table 1 and demonstrate their comparability with lot number 001503, with no significant differences found.



Representative FESEM Field at 20,000x magnification

Recommended Use of the Adenoviral Reference Material

In the U.S., FDA-CBER has requested that sponsors use the Adenovirus Reference Material (ARM) to establish the infectious titer and particle concentration of a laboratory's internal adenovirus reference preparation, and, then, to subsequently validate sponsor particle concentration and infectious titer assays so that units reported for the adenovirus product correspond in meaning to the ARM [2]. Values for adenovirus infectious titer are particularly dependent on the assay method. The ARM is intended to provide a fixed reference point so that if the same preparation is assayed using different methods, the resulting adjusted titers (NAS infectious units/mL) will be similar. The number of replicates required to obtain an accurate particle concentration or infectious titer will depend on the variance of the assays being used.

The ARM could be used to validate the methods a laboratory uses to determine particle concentration and infectious titer. The ARM could also be used to validate the infectious titer of the positive control virus used in replication-competent adenovirus assays. However, on-going validation work should be performed using the laboratory's internal adenovirus reference material, as the availability of the ARM is limited. Sponsors of adenovirus-related INDs should consult with the FDA Center for Biologics for further guidance. However it is not the intent of the FDA to standardize assay methods across the field or to require that the values assigned to the Adenovirus Reference Material be duplicated during validation studies [2]. There is no requirement in the U.S. to follow ARMWG procedures when assaying the particle concentration or infectious titer.

In September 2002 the ICH Gene Therapy Discussion Group recommended that sponsors in all three regions use the ARM to establish infectious titer and particle concentration assays for their adenovirus products. Health authorities in the three regions agreed to collect information and review it at a later date [12].

Recommended Host Cells

The ARM was prepared using a working cell bank of HEK 293 cells reserved for this project. It is not recommended that laboratories use the ARM to produce adenovirus. However, for the purposes of assay development, the user should be aware that the virus can be propagated on a variety of cells. The end-user must assess how an assay is impacted by the nature and characteristics of the host cells selected. HEK 293 cells that support replication of adenovirus are available from ATCC as CRL-1573.

Manufacture of the Adenovirus Reference Material

The original source for the ARM is a plaque isolate from a serially plaqued sample of Adenovirus, Type 5 (VR-5, ATCC). Plaque purification was performed on certified A549 cells (ATCC CCL-185). The plaque isolate was minimally amplified and found to be sterile, as well as free of mycoplasma and adeno-associated virus. The amplified plaque isolate was used to manufacture a Virus Bank. The adenovirus was cultured on microcarriers seeded with HEK 293 cells from a certified Working Cell Bank. The Virus Bank (VR-1517) was certified to be free of adventitious agents, and was used to produce the Adenovirus Reference Material. The ARM was produced by culturing on HEK 293 cells from the Working Cell Bank in Cell Cubes®, and purified using a single-column chromatography process [13], an anion exchange resin. The purified ARM was formulated, sterile filtered, and divided into 4 bulk sub-lots. Each sub-lot was sterile filtered and filled on a separate day, and was put onto a long-term stability program. The manufacture of the ARM and of the Virus Bank was performed under CGMP with full documentation. All bovine serum and trypsin used during these processes was certified as to country of origin and to be free of adventitious agents.

Recommended Storage and Stability Information

Short-term field use and shipping configuration studies were performed [14]. The field use study focused on the impact of multiple freeze-thaw cycles, thaw and subsequent storage at 2-8ºC over 7 days, and thaw and subsequent storage at room temperature over 7 days. Stability studies on the ARM have shown aggregation in some vials after a single freeze-thaw, *i.e.*, after standing for more than 4 hours upon thaw after receipt from ATCC. The ARM can be thawed at room temperature and left at either room temperature or at 2-8°C for as long as 4 hours without impact.

The shipping stability study examined the impact on the ARM when packaged on dry ice using the ATCC shipping configuration, with the package held for two days at 40ºC and then an additional day at 50ºC. Data confirmed that the ARM could be successfully shipped in the ATCC long distance configuration.

Long-term stability was established in a five-year stability study that initiated in 2001. The long-term stability study monitored the ARM when stored frozen at -80ºC, and, on a more limited basis, when frozen at -80ºC but stored long-term at -20ºC. Stability was evaluated particle quality and concentration as determined by an anion exchange HPLC assay [15] and for activity by an infectious titer assay incorporating the NAS calculation (FACS-based infectious titer assay using hexon expression as the endpoint). At various time points stability was also assessed via particle concentration by A 260nm in SDS, particle size by photon correlation spectroscopy, particle aggregation by ratio of A 320nm to A 260nm (without SDS), and a qualitative assessment of micro-aggregation via field emission scanning electron microscopy and assessment of sterility as a correlative for container integrity. Data is posted on The International Society for BioProcess Technology website http://www.isbiotech.org/ and can also be found in Table 2. Data through the 108 month time point demonstrate that the ARM is stable stored frozen at -80°C. Long-term storage at -20°C, however, is not recommended as both particle concentration and infectious titer drop significantly by 12 months; other parameters also indicate a significant change has taken place (Table 3).

Table 1. Comparison of ARM Sublots.

Assay	Time Point (Month)	Lot No. 001503	Lot No. 001504	Lot No. 001505	Lot No. 001506
Particles per mL via ResQ HPLC [15]	0	6.29×10^{11}	6.48×10^{11}	6.30×10^{11}	6.71×10^{11}
	12	5.44×10^{11}	5.50×10^{11}	5.52×10^{11}	5.57×10^{11}
	36	5.00×10^{11}	5.04×10^{11}	5.18×10^{11}	5.17×10^{11}
Particles per mL via OD260nm / SDS ARMWG SOP	0	6.34×10^{11}	6.51×10^{11}	6.28×10^{11}	6.68×10^{11}
	12	6.07×10^{11}	5.87×10^{11}	5.90×10^{11}	6.03×10^{11}
	36	5.04×10^{11}	5.63×10^{11}	5.31×10^{11}	5.30×10^{11}
Infectious Titer (FACS NAS IU/mL)	0	3.4×10^{11}	2.5×10^{11}	2.5×10^{11}	2.2×10^{11}
	12	3.6×10^{11}	2.9×10^{11}	2.9×10^{11}	2.2×10^{11}
Particle Size (nm)	0	112.6	115.3	112.5	113.0
	12	112.7	114.4	114.6	110.5
Virus Aggregation via OD 320nm / 260nm	0	0.22	0.21	0.21	0.22
	12	0.24	0.22	0.26	0.22

Table 2. Stability Data at -80°C for Lot Nos. 001503, 001504, 001505, and 001506. Starting at 74 months, stability data were generated by different labs.

Assay	Time Point (Month)	Lot No. 001503	Lot No. 001504	Lot No. 001505	Lot No. 001506
Particles/mL via ResQ HPLC [15]	0 months (Aug 2001)	6.29×10^{11}	6.48×10^{11}	6.30×10^{11}	6.71×10^{11}
	6 months (Feb 2002)	5.41×10^{11}	Not performed	Not performed	Not performed
	9 months (May 2002)	5.32×10^{11}	Not performed	Not performed	Not performed
	12 months (Aug 2002)	5.44×10^{11}	5.50×10^{11}	5.52×10^{11}	5.57×10^{11}
	18 months (Feb 2003)	5.34×10^{11}	Not performed	Not performed	Not performed
	24 months (Aug 2003)	5.24×10^{11}	Not performed	Not performed	Not performed
	36 months (Jul 2004)	5.00×10^{11}	5.04×10^{11}	5.18×10^{11}	5.17×10^{11}
	50 months (Sep 2005)	5.10×10^{11}	5.21×10^{11}	5.17×10^{11}	5.18×10^{11}
	60 months (Aug 2006)	5.13×10^{11}	5.05×10^{11}	5.11×10^{11}	5.09×10^{11}
	74 months (Oct 2007)	6.5×10^{11}	6.7×10^{11}	5.9×10^{11}	6.8×10^{11}
Particles/mL via OD260nm / SDS ARMWG SOP	108 months (June 2010)	Not performed	5.84×10^{11}	Not performed	Not performed
Infectious Titer (FACS NAS IU/mL)	0 months (Aug 2001)	3.4×10^{11}	2.5×10^{11}	2.5×10^{11}	2.2×10^{11}
	6 months (Feb 2002)	2.4×10^{11}	Not performed	Not performed	Not performed
	9 months (May 2002)	1.1×10^{11}	Not performed	Not performed	Not performed
	12 months (Aug 2002)	3.9×10^{11}	2.9×10^{11}	2.9×10^{11}	2.2×10^{11}
	18 months (Feb 2003)	1.7×10^{11}	Not performed	Not performed	Not performed
	24 months (Aug 2003)	5.2×10^{11}	Not performed	Not performed	Not performed
	36 months (Jul 2004)	[a]Not available	Not performed	Not performed	Not performed
	50 months (Sep 2005)	4.3×10^{11}	Not performed	Not performed	Not performed
	60 months (Aug 2006)	4.2×10^{11}	Not performed	Not performed	Not performed
Infectious Titer , IU/mL (96-well format CPE method)	74 months (Oct 2007)	1.1×10^{11}	1.7×10^{11}	7.6×10^{10}	9.8×10^{10}
	107 months (May 2010)	Not performed	2.6×10^{11}	Not performed	Not performed

a: Assay not valid.

Table 3. Stability Data at -20°C for Lot No. 001503.

Assay	Time Point (Month)	Lot No. 001503
Particles/mL via ResQ HPLC [15]	0 months (Sep 2001)	6.29×10^{11}
	12 months (Nov 2002)	$<3.09 \times 10^{10}$ 3.37×10^{10}
	24 months (Nov 2003)	$<3.09 \times 10^{10}$ 3.37×10^{10}
Particles/mL via OD 260nm / SDS ARMWG SOP	0 months (Sep 2001)	6.34×10^{11}
	12 months (Nov 2002)	4.39×10^{11}
	24 months (Nov 2003)	4.02×10^{11}
Infectious Titer (FACS NAS IU/mL)	0 months (Sep 2001)	3.4×10^{11}
	12 months (Nov 2002)	$<2.4 \times 10^{7}$ $<9.0 \times 10^{0}$
	24 months (Nov 2003)	8.7×10^{8} $<2.4 \times 10^{7}$
Particle Size (nm)	0 months (Sep 2001)	112.6
	12 months (Nov 2002)	120.0
Virus Aggregation via OD 320nm / 260nm	0 months (Sep 2001)	0.22
	12 months (Nov 2002)	0.27
	24 months (Nov 2003)	0.43
Bioburden (Vial integrity surrogate)	0 months (Sep 2001)	Negative
	15 months (Dec 2002)	Negative

Comments

During the characterization phase, it was observed that all vials contained an organic contaminant that was presumed to be a plastic leachate. The contaminant can be detected as an early eluting peak by RP-HPLC [16, 17] with a distinct peak UV spectrum that distinguishes it from virus protein peaks. The contaminant is present in all four sub-lots. Its absorbance characteristics do not interfere with the A 260nm/SDS method used to determine particle concentration (contribution less than 2%).

A small group of laboratories determined particle concentration [18] and infectious titer using a variety of orthogonal, or alternative, methods. Data pertaining to these assays are posted at http://www.isbiotech.org/. Publications are included in the reference list where authors discuss use of the ARM [references numbered 19 or higher].

References:

All information regarding the development and characterization of the Adenovirus Reference Material can be found on The International Society for BioProcess Technology website http://www.isbiotech.org/. The ARMWG has published many of the details in the scientific literature.

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Organizations and Laboratories That Donated Services and/or Materials, Participated in the Characterization Phase, or Otherwise Made Substantial Contributions Through Their Participation in the ARMWG

(Listed alphabetically)

AFSSAPS (Lyons, France)
Althea Technologies, Inc. (San Diego, CA)
American Type Culture Collection (Manassas, VA)
Amersham Biosciences (Piscataway, NJ)
AppTec Laboratory Services (Camden, NJ)
Berlex Biosciences (Richmond, CA)
BioReliance Corporation (Rockville, MD)
Biogen, Inc. (Cambridge, MA)
Biotechnology Research Institute, NRC (Montreal, Quebec, Canada)
Callahan Associates Inc. (La Jolla, CA)
Canji, Inc. (San Diego, CA)
Cell Genesys, Inc. (Foster City, CA)
Cobra Therapeutics, (Keele, England, UK)

Corning, Inc. (Corning, NY)
Covance Laboratories, Inc. (Harrogate, England, UK)
Crucell N.V. (Leiden, The Netherlands)
EM Industries, Inc. (Gibbstown, NJ)
Food and Drug Administration / Center for Biologics Evaluation and Research (FDA/CBER) – (Rockville, MD)
Genetic Therapy Inc / Novartis (Gaithersburg, MD)
GenVec, Inc. (Gaithersburg, MD)
Genzyme Corporation (Framingham, MA)
Harvard Gene Therapy Initiative, Harvard Medical School (Boston, MA)
HyClone Laboratories, Inc. (Logan, UT)
Introgen Therapeutics, Inc. (Houston, TX)
Invitrogen Corporation (Grand Island, NY)
MDS Pharma Services (Bothell, WA)
National Institute for Biological Standards and Controls [NIBSC] (South Mimms, England, UK)
Nalge Nunc International (Rochester, NY)
Onyx Pharmaceuticals, Inc. (Richmond, CA)
Pall Life Sciences, Inc. (Northborough, MA)
Q-BIOgene, Inc. (Montreal, Quebec, Canada)
Q-One Biotech, Ltd. (Worcester, MA and Glasgow, Scotland, UK)
Sajjadi Associates (Encinitas, CA)
Schering Plough Research Institute (Union, NJ)
Selective Genetics, Inc. (San Diego, CA)
SeqWright, Inc. (Houston, TX)
Stedim, Inc. (Concord, CA)
Texcell – Institut Pasteur (Paris, France)
Transgene S.A. (Strasbourg, France)
University of Alabama at Birmingham, Vector & Vaccine Production Facility (Birmingham, AL)
University of Texas at Austin – Croyle Laboratory (Austin, TX)
The Williamsburg BioProcessing Foundation (Virginia Beach, VA)

Biosafety Level 2

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Appropriate safety procedures should always be used with this material. See the National Institutes of Health publication, **Guidelines for Research Involving Recombinant DNA Molecules**. Detailed discussions of laboratory safety procedures are provided in the U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories**, Centers for Disease Control (1999), Human Health Service Publication No. (CDC) 939-8395, U.S. Dept. of Health and Human Services, 4th Edition, U.S. Government Printing Office, Washington, D.C. This information is available in its entirety in the Center for Disease Control Office of Health and Safety's website at http://www.cdc.gov/od/ohs or at the NIH website at http://www.nih.gov/od/ors/ds/pubs/bmbl/contents.htm.

Use Restrictions

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Key Abbreviations Used on this Product Sheet

ARMWG – Adenovirus Reference Material Working Group
ATCC – American Type Culture Collection
BSA – bovine serum albumin
FACS – flow cytometry
FDA - CBER – Food and Drug Administration - Center for Biologics Evaluation and Research
FESEM – Field emission scanning electron microscopy
IND – Investigational New Drug (application to FDA)
NAS – Normalized and Adjusted Standard
NIH – National Institutes of Health, U.S.
NIU/mL – NAS infectious units per milliliter
RCA – replication competent adenovirus
ResQ HPLC – Resource Q anion exchange high pressure liquid chromatography assay
RP-HPLC – reverse phase high pressure liquid chromatography

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